https://doi.org/10.17221/177/2022-HORTSCI

# Improving photosynthesis and the ascorbate-glutathione cycle of own-root and grafted-root chrysanthemums by brassinolide under drought stress

*Peng Ming Yang[1]*, Rui Iao Yang[2], Song Tao He[3]*

[1]*College of Horticulture and Landscape Architecture, Henan Institute of Science and Technology, Xinxiang, P. R. China*
[2]*College of Life Science and Biotechnology, Heilongjiang Bayi Agricultural University, Daqing, P. R. China*
[3]*Agricultural College, Sumy National Agrarian University, Sumy, Ukraine*

**Corresponding author: yangpengming@126.com*

**Citation:** Yang P.M., Yang R.J., He S.T. (2024): Improving photosynthesis and the ascorbate-glutathione cycle of own-root and grafted-root chrysanthemums by brassinolide under drought stress. Hort. Sci. (Prague), 51: 59–67.

**Abstract:** Many studies have demonstrated that brassinolide improves the drought tolerance of plants. This study aims to test whether the drought tolerance of chrysanthemums can be improved by brassinolide and to clarify the underlying physiological mechanism. An own-root chrysanthemum and a corresponding grafted-root line (*Artemisia annua* rootstock) were treated with brassinolide under three water levels in a randomised complete block design with five replications. The results showed that brassinolide increased the relative water content, net photosynthetic rate, chlorophyll (Chl) fluorescence parameters, Rubisco, ascorbate peroxidase, glutathione reductase, dehydroascorbate reductase and monodehydroascorbate reductase activities, ascorbate/dehydroascorbate, glutathione/oxidised glutathione and dry mass, and reduced the $H_2O_2$ content in the own-root and grafted-root chrysanthemums, especially under drought stresses. The magnitude of the changes to the parameters was greater in the own-root line than in the corresponding grafted-root line under brassinolide treatment. The above parameters showed significant differences ($P < 0.05$) between the brassinolide chrysanthemums and the corresponding non-brassinolide chrysanthemums under drought stresses. This might be the physiological mechanism of improved drought tolerance by brassinolide in chrysanthemums.

**Keywords:** *Artemisia annua* rootstock; relative water content; Rubisco activity; $H_2O_2$; dry mass

The chrysanthemum (*Chrysanthemum morifolium*) has been cultivated worldwide, is widely used as a cut flower, potted plant, and in landscaping too, second only to the rose in terms of its market value (Guan et al. 2017). The chrysanthemum often suffers from a broad range of environmental stresses, *e.g.*, cold, heat, drought, and high salinity. As one of the major adverse environmental stresses that hinder plant growth worldwide, the threat of drought stress is increasing due to the adverse global climate changes (Teuling 2018). Photosynthesis is very susceptible to environmental changes and is often the first process that is affected by drought stress. Drought stress decreases the internal $CO_2$ concen-

Supported by Henan Institute of Science and Technology, P. R. China, Project No. 20703028.

https://doi.org/10.17221/177/2022-HORTSCI

tration, inhibits the Rubisco activity and adenosine triphosphate (ATP) synthesis, and leads to a decrease in the net photosynthetic rate ($P_N$) (Zhang et al. 2021). Moreover, drought stress causes oxidative damage via the increasing accumulation of reactive oxygen species (ROS), which reduces photosynthesis, alters the activities of enzymes and causes cell membrane damage (Dias, Brüggemann 2007). To decrease these damages, plants have evolved different pathways, such as increasing the antioxidant compounds, either the non-enzymatic or enzymatic ones. The ascorbate-glutathione (AsA-GSH) cycle may have an important role in maintaining the cell redox status in plants, especially under abiotic stress. AsA and GSH are major non-enzymatic antioxidants, while ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) have essential roles in the AsA-GSH cycle (Hu, Shan 2018; Sadura, Janeczko 2018).

Grafting is widely used to improve the abiotic/biotic stress tolerance of many types of plants (Huang et al. 2016, Sun et al. 2020). Previous research has suggested that the herbaceous genus *Artemisia* provides a useful rootstock for enhancing the abiotic/biotic stress tolerance in chrysanthemums (Chen et al. 2018; Zhang et al. 2019). Furthermore, treatment with a growth regulator has been demonstrated to be an effective strategy in improving plant tolerance to environmental stresses (Hu et al. 2013; Sang et al. 2016). As a plant growth regulator, brassinolide (BL) is used to increase the growth and quality of cultivated plants (Hu et al. 2013), and enhance the plant's tolerance to a variety of abiotic and biotic stresses (Tanveer et al. 2018; Li et al. 2020). However, the influence of BL on the photosynthesis and AsA-GSH cycle in grafted-root and own-root chrysanthemums under drought stress is still unclear. This study aims to investigate whether BL can improve the drought tolerance of grafted-root and own-root chrysanthemums and to clarify the underlying physiological mechanism.

## MATERIAL AND METHODS

**Material.** In this experiment, the commercial *Artemisia annua* rootstock cultivar 'Bai121' and landscaping type chrysanthemum cultivar 'FY' (*Chrysanthemum morifolium*) were used. The brassinolide (Sigma, USA) was prepared as an ethanolic solution, and distilled water was added until a 0.06 mol/L concentration was obtained. An equal amount of ethanol was added to the controls.

**Experimental design.** The experiment was carried out in a rain shelter greenhouse under natural daylight conditions in Xinxiang, China (35°18'N, 113°52'E) from March to August 2021. The seeds of *Artemisia annua* were sown in plastic pots (30 cm in diameter × 25 cm in depth) filled with a 1 : 1 : 1 (v/v/v) mixture of peat, perlite, and vermiculite. The chrysanthemum cultivar 'FY' was propagated by stem cuttings and grown in plastic pots (30 cm in diameter × 25 in depth) filled with a 1 : 1 : 1 (v/v/v) mixture of peat, perlite, and vermiculite. On May 1st, healthy *Artemisia annua* plants 30 cm in height and with a 6-mm stem diameter were used as the rootstocks, and apical shoots with a length of 15 cm and a stem diameter of 4 mm from healthy chrysanthemums were used as the scions. Insert grafting was performed as described by Lee (1994). In order to maintain high humidity, the grafted seedlings were covered with a layer of transparent plastic film, and the grafted seedlings were placed in the shade for 72 hours. The plastic film was removed for a short time during the initial days to control the relative humidity, and it was completely removed after 10 days of grafting. The pots were watered to soil saturation every day and a multi-purpose fertiliser was applied every week together with irrigation. After 30 days of grafting, morphologically uniform grafted-root and own-root chrysanthemums were selected for the drought stress and BL treatments. The drought treatment and BL treatment were carried out at the same time. Drought stress was imposed by withholding water from the plots until the soil water potential was achieved. During the test, the three levels of soil water potential were 80% of field capacity (–0.075 MPa, CK), 60% of field capacity (–0.140 MPa) and 40% of field capacity (–0.380 MPa). The soil water potential was measured by a pressure plate apparatus (Shimadzu, CL-800, Kyoto, Japan) and the amount of water loss was supplied to each plot to maintain the intended soil water content. A brassinolide solution with concentration of 0.06 mol/L was sprayed at 7:00–8:00 in the morning until the solution slid off the leaf surface. All the treatments were arranged in a randomised complete block design with five replications. Each replicate line was comprised of five rows, and each row had five individuals. The spacing between the rows/individuals was 50 cm. The treat-

https://doi.org/10.17221/177/2022-HORTSCI

ments were: (1) well-watered (–0.075 MPa) and non-BL; (2) well-watered (–0.075 MPa) and sprayed with BL; (3) –0.140 MPa drought stress and non-BL; (4) –0.140 MPa drought stress and sprayed with BL; (5) –0.380 MPa drought stress and non-BL; (6) –0.380 MPa drought stress and sprayed with BL. Five individual plants were chosen randomly from each line of each treatment at day 10 after the drought stress and BL treatments and were used for testing the relevant parameters. The $P_N$ and Chl fluorescence parameters were measured on the uniform third fully expanded leaf (from the top). The flesh samples (fully-expanded leaves) were collected and used for testing the AsA/DHA and GSH/GSSG ratios, $H_2O_2$ content and enzyme activity. The dry mass was determined in August.

**Relative water content (RWC).** The relative water content was determined on the fresh third fully expanded leaf (from the top) discs measuring 2 cm in diameter. The RWC was calculated according to Hayat et al. (2007). Each result shown is the mean of 5 replicated treatments.

**Net photosynthetic rate ($P_N$).** The LI6400 portable photosynthesis system (LI-COR co., USA) was used to measure the $P_N$ of the third leaf (from the top) at 10:00 in a day. Each result shown is the mean of 5 replicated treatments.

**Chl fluorescence parameters.** The Chl fluorescence parameters were measured on the uniform third fully expanded leaf (from the top) using a portable chlorophyll fluorometer (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany) under natural environmental conditions, on sunny days with air temperatures about 28 °C with a relative humidity about 70%, between 9:00 and 11:00 hours. The mean values of the leaf maximum photochemical efficiency of PSII ($F_v/F_m$) and the actual photochemical efficiency of PSII ($\Phi_{PSII}$) were calculated according to Baker (2008). Each result shown is the mean of 5 replicated treatments.

**The $H_2O_2$ content.** The hydrogen peroxide content was measured spectrophotometrically after reaction with potassium iodide (KI) as described by Liu et al. (2018). Each result shown is the mean of 5 replicated treatments.

**Rubisco (EC. 4.1.1.39) activity (initial).** Rubisco was extracted according to Chen et al. (2005) and the Rubisco activity was assayed according to Cheng and Fuchigami (2000). The sample extract (50 µL) was added to a cuvette containing 900 µL of an assay solution, immediately followed by adding 50 µL of 10 mM RuBP, then mixing well. The change in absorbance at 340 nm was monitored for 40 s. The assay solution contained 100 mM HEPES-KOH (pH 8.0), 25 mM $KHCO_3$, 20 mM $MgCl_2$, 3.5 mM ATP, 5 mM phosphocreatine, 5 units NAD-glyceraldehyde-3-phosphate dehydrogenase (NAD-GAPDH, EC 1.2.1.12), 5 units 3-phosphoglyceric phosphokinase (PCK, EC 2.7.2.3), 17.5 units creatine phosphokinase (EC 2.7.3.2), 0.25 mM NADH, 0.5 mM RuBP, and 50 µL sample extract. Each result shown is the mean of 5 replicated treatments.

**The the activity of dehydroascorbate reductase (DHAR, EC 1.8.5.1), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), glutathione reductase (GR, EC 1.8.1.7) and ascorbate peroxidase (APX, EC 1.11.1.11).** The DHAR, MDHAR, GR and APX activities were measured according to Noctor et al. (2016). Each result shown is the mean of 5 replicated treatments.

**Ascorbate (AsA)/dehydroascorbate (DHA) and glutathione (GSH)/oxidised glutathione (GSSG) analysis.** The ascorbate and DHA contents were measured according to Ueda et al. (2013) and the ratio between the AsA content and the DHA content was expressed as AsA/DHA. The GSSG and GSH contents were measured according to Rao and Ormrod (1995) and the ratio between the GSH content and the GSSG content was expressed as GSH/GSSG. Each result shown is the mean of 5 replicated treatments.

**Dry mass per plant.** An electronic balance was used to analyse the dry mass (g). The mean value was derived from 5 sample plants.

**Statistical analysis.** All the data in the present study were expressed as the Mean ± SD. The analysis of the significance was performed using SAS software (SAS Institute, Inc., Cary, NC, USA). A three-way analysis of variance (*ANOVA*) method (*Tukey's* multiple range test) was used to detect the significance ($P < 0.05$).

## RESULTS

This experiment showed that the RWC, $P_N$, $F_v/F_m$, $\Phi_{PSII}$ and Rubisco activity of all the chrysanthemums decreased with the reduced soil water content (Table 1). The drought stress (DS) significantly ($P < 0.05$) reduced the RWC, $P_N$, $F_v/F_m$, $\Phi_{PSII}$ and Rubisco activity and the magnitude of the decrease in these parameters were greater in the own-root line than

https://doi.org/10.17221/177/2022-HORTSCI

Table 1. The RWC (%), PN (μmol/m$^2$.s), Fv/Fm, ΦPSII and Rubisco activity (μmol/min.g) of the own-root and grafted-root chrysanthemums under different water treatments (mean ± SD)

| Traits | Water levels | O | BL-O | G | BL-G |
|---|---|---|---|---|---|
| RWC | –0.075 MPa | 93.3 ± 4.6$^a$ | 94.1 ± 4.1$^a$ | 94.5 ± 3.9$^a$ | 94.8 ± 4.2$^a$ |
| | –0.140 MPa | 70.6 ± 2.2$^b$ | 79.3 ± 3.2$^c$ | 80.7 ± 2.5$^c$ | 87.6 ± 2.5$^d$ |
| | –0.380 MPa | 57.3 ± 1.7$^e$ | 68.8 ± 1.6$^b$ | 71.6 ± 2.1$^b$ | 82.3 ± 2.8$^c$ |
| $P_N$ | –0.075 MPa | 6.32 ± 0.27$^a$ | 7.28 ± 0.32$^b$ | 8.52 ± 0.37$^c$ | 9.71 ± 0.39$^d$ |
| | –0.140 MPa | 4.81 ± 0.18$^e$ | 5.86 ± 0.21$^f$ | 7.35 ± 0.29$^b$ | 8.64 ± 0.35$^c$ |
| | –0.380 MPa | 1.83 ± 0.07$^g$ | 3.12 ± 0.10$^h$ | 4.56 ± 0.18$^e$ | 5.92 ± 0.23$^f$ |
| $F_v/F_m$ | –0.075 MPa | 0.76 ± 0.028$^a$ | 0.82 ± 0.035$^b$ | 0.83 ± 0.036$^b$ | 0.88 ± 0.037$^c$ |
| | –0.140 MPa | 0.60 ± 0.022$^d$ | 0.71 ± 0.026$^e$ | 0.73 ± 0.031$^{ae}$ | 0.81 ± 0.032$^b$ |
| | –0.380 MPa | 0.42 ± 0.013$^f$ | 0.53 ± 0.018$^g$ | 0.57 ± 0.022$^d$ | 0.66 ± 0.029$^h$ |
| $\Phi_{PSII}$ | –0.075 MPa | 0.50 ± 0.021$^a$ | 0.57 ± 0.023$^{bc}$ | 0.59 ± 0.025$^b$ | 0.63 ± 0.028$^d$ |
| | –0.140 MPa | 0.39 ± 0.013$^e$ | 0.47 ± 0.016$^f$ | 0.51 ± 0.019$^a$ | 0.55 ± 0.022$^c$ |
| | –0.380 MPa | 0.31 ± 0.011$^g$ | 0.39 ± 0.012$^e$ | 0.42 ± 0.015$^h$ | 0.46 ± 0.018$^f$ |
| Rubisco activity | –0.075 MPa | 0.75 ± 0.025$^a$ | 0.87 ± 0.032$^b$ | 0.99 ± 0.032$^c$ | 1.06 ± 0.037$^d$ |
| | –0.140 MPa | 0.50 ± 0.018$^e$ | 0.60 ± 0.021$^f$ | 0.70 ± 0.025$^g$ | 0.79 ± 0.028$^a$ |
| | –0.380 MPa | 0.18 ± 0.007$^h$ | 0.27 ± 0.011$^i$ | 0.38 ± 0.013$^j$ | 0.48 ± 0.016$^e$ |

$^{a–j}$Different letters are significantly different ($P$ < 0.05) in each trait

RWC – relative water content; $P_N$ – net photosynthetic rate; $F_v/F_m$ – maximum photochemical efficiency of PSII; ΦPSII – actual photochemical efficiency of PSII; G – grafted-root chrysanthemum; O – own-root chrysanthemum; BL – brassinolide

in the grafted-root line. The RWC, $P_N$, $F_v/F_m$, $\Phi_{PSII}$ and Rubisco activity of the own-root line decreased by 24.3%, 23.9, 20.9%, 23.1% and 34.0% (–0.140 MPa), and 38.6%, 71.0%, 45.5%, 38.0% and 75.7% (–0.380 MPa), respectively, while those of grafted-root line decreased by 14.6%, 13.7%, 12.0%, 14.8% and 29.3 % (–0.140 MPa), and 24.2%, 46.5%, 31.0%, 30.0% and 61.3% (–0.380 MPa), respectively. Brassinolide improved the RWC, $P_N$, $F_v/F_m$, $\Phi_{PSII}$ and Rubisco activity in all the chrysanthemums under the well-watered (WW) and DS treatments (Table 1), and the improvement in the magnitude of these parameters was greater in the own-root line than in the grafted-root line. The RWC, $P_N$, $F_v/F_m$, $\Phi_{PSII}$ and Rubisco activity of the own-root line increased by 0.86%, 15.2%, 8.0%, 12.9% and 15.3% (–0.075 MPa), 12.3%, 21.8%, 17.1%, 22.0% and 21.8% (–0.140 MPa), and 20.1%, 70.5%, 28.0%, 23.7% and 44.8% (–0.380 MPa), respectively, while those of the grafted-root line increased by 0.31%, 14.0%, 6.3%, 6.9% and 7.7% (–0.075 MPa), 8.6%, 17.6%, 10.4%, 9.3% and 12.9% (–0.140 MPa), and 14.9%, 29.8%, 15.5%, 11.8% and 26.8% (–0.380 MPa), respectively.

Drought stress significantly increased the $H_2O_2$ content compared with the WW condition (Table 2). The $H_2O_2$ content of all the chrysanthemums increased when the drought stress increased (Table 2), and the increase in the magnitude of the $H_2O_2$ content was more apparent in the own-root line than in the grafted-root line. The $H_2O_2$ content of the own-root line increased by 74.9% (–0.140 MPa) and 209.6% (–0.380 MPa), while that of grafted-root line increased by 46.6% (–0.140 MPa) and 120.9% (–0.380 MPa). Brassinolide reduced the $H_2O_2$ con-

Table 2. The $H_2O_2$ content (μmol/g) of the own-root and grafted-root chrysanthemums under different water treatments (mean ± SD)

| Water levels | O | BL-O | G | BL-G |
|---|---|---|---|---|
| –0.075 MPa | 7.92 ± 0.26$^a$ | 7.56 ± 0.22$^a$ | 6.98 ± 0.23$^b$ | 6.75 ± 0.19$^b$ |
| –0.140 MPa | 13.85 ± 0.49$^c$ | 12.15 ± 0.47$^d$ | 10.23 ± 0.38$^e$ | 9.25 ± 0.30$^f$ |
| –0.380 MPa | 24.52 ± 0.88$^g$ | 19.46 ± 0.75$^h$ | 15.42 ± 0.61$^i$ | 13.42 ± 0.48$^c$ |

$^{a–i}$Different letters are significantly different ($P$ < 0.05)

O – own-root chrysanthemum; G – grafted-root chrysanthemum; BL – brassinolide

https://doi.org/10.17221/177/2022-HORTSCI

tent in the WW lines, but did not significantly affect the parameter. Meanwhile, BL significantly reduced the $H_2O_2$ content in the DS lines (Table 2). Additionally, BL decreased the $H_2O_2$ content more dramatically in the own-root line than in the grafted-root line under all the water conditions (Table 2). The $H_2O_2$ content of the own-root line decreased by 4.5% (–0.075 MPa), 12.3% (–0.140 MPa) and 20.6% (–0.380 MPa), while that of the grafted-root line decreased by 3.3% (–0.075 MPa), 9.6% (–0.140 MPa) and 13.0% (–0.380 MPa).

Brassinolide improved the APX, MDHAR and DHAR activities of all the chrysanthemums under the WW and DS treatments (Table 3), and the improvement in the magnitude of these parameters was greater in the own-root line than in the grafted-root line. The drought stress significantly decreased the GR activity compared with the WW treatment (Table 3). The decrease in the magnitude of the GR activity was greater in the own-root line than in the grafted-root line. Brassinolide improved the GR activity in all the chrysanthemums under the WW and DS conditions (Table 3), and the improvement in the magnitude of the parameter was greater in the own-root line than in the grafted-root line. Moreover, the APX, GR, MDHAR and DHAR activities of the grafted-root line were significantly ($P$ < 0.05) higher than those of the own-root line under the DS conditions (Table 3).

The drought stress significantly decreased the AsA/DHA, GSH/GSSG and dry mass of all the chrysanthemums (Table 4). However, the DS decreased the AsA/DHA, GSH/GSSG and dry mass more dramatically in the own-root line than in the grafted-root line. The AsA/DHA, GSH/GSSG and dry mass of the own-root line decreased by 73.8%, 80.0 % and 37.5 % (–0.140 MPa), and 87.8%, 91.3% and 52.4% (–0.380 MPa), respectively, while those of grafted-root line decreased by 61.6%, 64.0 % and 21.2% (–0.140 MPa), and 77.1%, 79.4% and 40.0% (–0.380 MPa), respectively. Brassinolide improved the AsA/DHA, GSH/GSSG and dry mass of all the chrysanthemums under the three water levels (Table 4), and the improvement in the magnitude of these parameters was greater in the own-root line than in the grafted-root line. The AsA/DHA, GSH/GSSG and dry mass of the own-root line increased by 8.3%, 9.3% and 14.4% (–0.075 MPa), 24.5%, 27.7% and 31.3% (–0.140 MPa), and 38.2%, 44.8% and 29.1% (–0.380 MPa), respectively, while those of grafted-root line increased by 5.4%, 6.4% and 8.3% (–0.075 MPa), 13.1%, 17.4% and 16.9% (–0.140 MPa), and 30.9%, 28.4% and 21.2% (–0.380 MPa), respectively.

Drought significantly affected the above parameters in all the chrysanthemums (Tables 1–4). Under mild drought (–0.140 MPa), the order of variation in the magnitude was GSH/GSSG, $H_2O_2$, AsA/DHA, APX, dry mass, GR, MDHAR, Rubisco, DHAR, RWC, $P_N$, $\Phi_{PSII}$ and $F_v/F_m$ in the own-root line, and APX, GSH/GSSG, AsA/DHA, MDHAR, $H_2O_2$, DHAR, Rubisco, GR, dry mass, $\Phi_{PSII}$, RWC,

Table 3. The APX, GR, MDHAR and DHAR activities (μmol/min g) of the own-root and grafted-root chrysanthemums under the different water treatments (mean ± SD)

| Traits | Water levels | O | BL-O | G | BL-G |
|---|---|---|---|---|---|
| APX | –0.075 MPa | $2.96 \pm 0.11^{a}$ | $3.17 \pm 0.12^{b}$ | $3.23 \pm 0.11^{b}$ | $3.42 \pm 0.12^{c}$ |
| | –0.140 MPa | $4.55 \pm 0.19^{d}$ | $5.17 \pm 0.19^{e}$ | $5.62 \pm 0.23^{f}$ | $6.07 \pm 0.26^{g}$ |
| | –0.380 MPa | $3.22 \pm 0.11^{b}$ | $4.32 \pm 0.16^{h}$ | $4.89 \pm 0.19^{i}$ | $5.41 \pm 0.22^{ef}$ |
| DHAR | –0.075 MPa | $34.62 \pm 1.23^{a}$ | $38.23 \pm 1.54^{b}$ | $39.53 \pm 1.29^{b}$ | $42.02 \pm 1.57^{c}$ |
| | –0.140 MPa | $43.53 \pm 1.87^{c}$ | $50.14 \pm 2.09^{d}$ | $52.84 \pm 2.13^{e}$ | $57.42 \pm 2.25^{f}$ |
| | –0.380 MPa | $18.65 \pm 0.69^{g}$ | $23.12 \pm 0.82^{h}$ | $27.45 \pm 1.04^{i}$ | $30.83 \pm 1.13^{j}$ |
| GR | –0.075 MPa | $0.94 \pm 0.032^{a}$ | $1.03 \pm 0.037^{b}$ | $1.09 \pm 0.039^{c}$ | $1.16 \pm 0.046^{d}$ |
| | –0.140 MPa | $0.61 \pm 0.021^{e}$ | $0.77 \pm 0.029^{f}$ | $0.81 \pm 0.028^{g}$ | $0.96 \pm 0.039^{a}$ |
| | –0.380 MPa | $0.32 \pm 0.011^{h}$ | $0.45 \pm 0.018^{i}$ | $0.49 \pm 0.017^{j}$ | $0.60 \pm 0.025^{e}$ |
| MDHAR | –0.075 MPa | $0.76 \pm 0.028^{a}$ | $0.82 \pm 0.032^{b}$ | $0.84 \pm 0.035^{bc}$ | $0.89 \pm 0.034^{c}$ |
| | –0.140 MPa | $1.02 \pm 0.039^{d}$ | $1.13 \pm 0.046^{e}$ | $1.33 \pm 0.056^{f}$ | $1.43 \pm 0.061^{g}$ |
| | –0.380 MPa | $0.64 \pm 0.023^{h}$ | $0.73 \pm 0.027^{a}$ | $0.75 \pm 0.028^{a}$ | $0.83 \pm 0.032^{b}$ |

[a–j]Different letters are significantly different ($P$ < 0.05) in each trait

O – own-root chrysanthemum; G – grafted-root chrysanthemum; BL – brassinolide, APX – ascorbate peroxidase; GR – glutathione reductase; MDHAR – monodehydroascorbate reductase; DHAR – dehydroascorbate reductase

https://doi.org/10.17221/177/2022-HORTSCI

Table 4. The AsA/DHA, GSH/GSSG and dry mass (g) of the own-root and grafted-root chrysanthemums under different water treatments (mean ± SD)

| Traits | Water levels | O | BL-O | G | BL-G |
|---|---|---|---|---|---|
| | –0.075 MPa | 1.08 ± 0.032[a] | 1.17 ± 0.041[b] | 1.22 ± 0.048[b] | 1.28 ± 0.052[c] |
| AsA/DHA | –0.140 MPa | 0.28 ± 0.011[d] | 0.35 ± 0.012[e] | 0.47 ± 0.017[f] | 0.53 ± 0.019[g] |
| | –0.380 MPa | 0.13 ± 0.004[h] | 0.18 ± 0.006[i] | 0.28 ± 0.009[d] | 0.36 ± 0.013[e] |
| | –0.075 MPa | 19.38 ± 0.73[a] | 21.16 ± 0.65[b] | 23.43 ± 0.94[c] | 24.94 ± 0.84[d] |
| GSH/GSSG | –0.140 MPa | 3.87 ± 0.11[e] | 4.94 ± 0.17[f] | 8.44 ± 0.32[g] | 9.91 ± 0.28[h] |
| | –0.380 MPa | 1.69 ± 0.04[i] | 2.45 ± 0.09[j] | 4.83 ± 0.18[f] | 6.20 ± 0.20[k] |
| | –0.075 MPa | 168.56 ± 6.27[a] | 192.82 ± 7.53[b] | 197.15 ± 7.53[b] | 213.52 ± 8.26[c] |
| Dry mass | –0.140 MPa | 105.38 ± 3.86[d] | 138.32 ± 4.92[e] | 155.36 ± 5.42[f] | 181.56 ± 7.33[g] |
| | –0.380 MPa | 80.25 ± 2.85[h] | 103.58 ± 3.86[d] | 118.23 ± 4.07[i] | 143.30 ± 5.19[e] |

[a–k]Different letters are significantly different ($P$ < 0.05) in each trait

O – own-root chrysanthemum; G – grafted-root chrysanthemum; BL – brassinolide; AsA – ascorbate; GSH – glutathione; DHA – dehydroascorbate; GSSG – oxidized glutathione

$P_N$ and $F_v/F_m$ in the grafted-root line; Under severe drought (–0.380 MPa), the order of variation in the magnitude was $H_2O_2$, GSH/GSSG, AsA/DHA, Rubisco, $P_N$, GR, dry mass, APX, DHAR, $F_v/F_m$, RWC, $\Phi_{PSII}$ and MDHAR in the own-root line, and $H_2O_2$, GSH/GSSG, AsA/DHA, Rubisco, GR, APX, $P_N$, dry mass, $F_v/F_m$, DHAR, $\Phi_{PSII}$, RWC and MDHAR in the grafted-root line.

## DISCUSSION

In the present study, the DS reduced the RWC, $P_N$, $F_v/F_m$, $\Phi_{PSII}$ and Rubisco activity in the own-root and grafted-root chrysanthemums (Table 1), and the results were in agreement with previous studies (Sun et al. 2013; Erb, Zarzycki 2018). However, the parameters of the own-root line were more sensitive to drought stress compared to the grafted-root line, resulting in a decrease in the magnitude of those parameters that was greater in the own-root line than in the grafted-root line (Table 1). As a rootstock, the root system of *Artemisia annua* is more vigorous than that of the chrysanthemum (Chen et al. 2018). We reasoned that the vigorous root of the *Artemisia annua* rootstock could enhance the efficiency in the uptake of water in the grafted-root chrysanthemum which contributed to alleviating the negative effect of the drought on the RWC of the chrysanthemum. As a result, the RWC values of the grafted-root line were higher than those of the own-root line under both the WW and DS conditions, especially under the DS conditions (Table 1). The higher RWC is beneficial in improving the $P_N$, $F_v/F_m$, $\Phi_{PSII}$ and Rubisco activity in the DS plants. Therefore, the $P_N$, $F_v/F_m$, $\Phi_{PSII}$ and Rubisco activities were up-regulated in the grafted-root chrysanthemum versus the own-root chrysanthemum under both the WW and DS conditions (Table 1). Furthermore, BL improved the RWC, $P_N$, $F_v/F_m$, $\Phi_{PSII}$ and Rubisco activity of all the chrysanthemums, especially under drought stress (Table 1). As a steroid hormone, BL ameliorates the thylakoid membranes and chloroplast ultrastructure in stressed plants. The correct structure and functioning of the thylakoid membranes, where electron carriers are located, are essential to maintain a high PSII efficiency (Janeczko et al. 2016). This may be the reason why BL could alleviate the detrimental effect of drought stress on the $F_v/F_m$ and $\Phi_{PSII}$ in the chrysanthemum. Moreover, a previous study reported that BL promotes the expression of the genes encoding Rubisco (Xia et al. 2009). The brassinolide application increased the Rubisco activity in the chrysanthemum, especially under drought stress (Table 1), indicating that the expression of the genes encoding Rubisco in the chrysanthemum may be up-regulated by BL. Moreover, BL has a positive effect on the osmotic maintenance and membrane integrity, which can alleviate the negative effect of the DS on the plant's RWC (Khamsuk et al. 2018). Therefore, the RWC, $P_N$, $F_v/F_m$, $\Phi_{PSII}$ and Rubisco activity were up-regulated in the BL line versus the corresponding non-BL line, especially under drought stress (Table 1). The improvement in these parameters is beneficial in improving the photosynthetic capacity, efficiency of light energy utilisation and carbon fixation in the BL line and alleviate the detrimental effect of the DS on the dry mass accumulation of the own-root

https://doi.org/10.17221/177/2022-HORTSCI

and grafted-root chrysanthemums (Table 4). Furthermore, the improvement in the magnitude of these parameters in the own-root line was relatively high compared to the grafted-root line. These results suggest that the own-root chrysanthemum and grafted-root chrysanthemum responded differently to BL. The discrepancy can be explained by the fact that the own-root chrysanthemum had lower values of these parameters than the grafted-root chrysanthemum, resulting in that the own-root chrysanthemum had a higher increase potential in these parameters under the BL treatment compared to the grafted-root chrysanthemum. On the other hand, abiotic stresses usually lead to the accumulation of reactive oxygen species (ROS), ROS induces damage to the constituent membrane elements by the accumulation of the products of lipid peroxidation, which cause oxidative damage to plants (Yousuf et al. 2017). Our results showed that the DS significantly increased the $H_2O_2$ content compared with the WW treatment and the increase in the parameter was more pronounced in the own-root line than the grafted-root line, resulting in that the $H_2O_2$ content of the grafted-root line was significantly lower than that of the own-root line under the drought stresses (Table 2). Brassinolide significantly reduced the $H_2O_2$ content in the DS chrysanthemums (Table 2), and the mechanism might be that BL could lead to the inactivation of excessive ROS in plants (Sadura, Janeczko 2018). According to many researchers, ROS, in addition to the damaging action on cellular components and structures, also plays a signalling role (Noctor et al. 2016). The excessive $H_2O_2$ in the chrysanthemum might act as a signalling molecule responded to the DS which then triggers the antioxidant system to cope with the DS caused by the excessive $H_2O_2$ accumulation. The ascorbate-glutathione (AsA-GSH) cycle is an important component of the antioxidant defence system in plants (Hu and Shan 2018). In this cycle, there are two non-enzymatic antioxidants, AsA and GSH, and four enzymes, including APX, MDHAR, DHAR and GR. Ascorbate peroxidase clears away the $H_2O_2$ by using the reduced AsA as an electron donor, which produces $H_2O$ and monodehydroascorbate (MDHA). The monodehydroascorbate is reduced to ASA by MDHAR or further oxidised to dehydroascorbate. Dehydroascorbate uses GSH as a substrate to produce ASA and oxidised glutathione (GSSG) under the catalysis of DHAR. Glutathione reductase reduces the GSSG to reduced GSH. Therefore, the AsA-GSH cycle has an important role in clearing away the $H_2O_2$. In the present study, the DS significantly attenuated the AsA-GSH cycle, and led to an oxidised redox status of AsA and GSH, as shown by the decline in the AsA/DHA and GSH/GSSG (Table 4). However, the *Artemisia annua* rootstock could significantly alleviate the detrimental effect of the DS on the AsA-GSH cycle due to the APX, MDHAR, DHAR and GR activities, the AsA/DHA and GSH/GSSG of the grafted-root chrysanthemum were significantly higher than those of the own-root chrysanthemum under the drought stresses (Tables 3–4). Previous studies reported that the rootstock can regulate the expression of some genes in the scion (Ahsan et al. 2019). It implies that the *Artemisia annua* rootstock up-regulate the expression of the genes encoding APX, MDHAR, DHAR and GR in the grafted-root chrysanthemum, which, in turn, facilitates the AsA-GSH cycle, especially under DS conditions. Previous studies have reported BL can regulate the gene expressions at transcriptional or post-transcriptional levels in some plants (Deng et al. 2007, Xia et al. 2009). In the present study, BL significantly promoted the activity of APX, MDHAR, DHAR and GR in the own-root and grafted-root chrysanthemums under drought stresses, and resulted in the reduced redox status of AsA and GSH, as shown by the increase in the AsA/DHA and GSH/GSSG (Tables 3–4). It may imply that BL promoted the expression of the genes encoding the APX, MDHAR, DHAR and GR in the chrysanthemum. Furthermore, the BL positive effect on the own-root line was relatively high compared to the grafted-root line, we reasoned that the APX**,** MDHAR, DHAR and GR activities, AsA/DHA and GSH/GSSG of the own-root chrysanthemum were significantly lower than those of grafted-root chrysanthemum (Tables 3–4), therefore, the up-regulation potential of these parameters might be greater in the own-root line than in the corresponding grafted-root line under the BL treatment.

## CONCLUSION

The DS significantly decreased the RWC, inhibited the $P_N$, $F_v/F_m$, $\Phi_{PSII}$, Rubisco activity and AsA-GSH cycle, and increased the $H_2O_2$ content, which led to a significant reduction in the dry mass of the own-root and grafted-root chrysanthemums. However, the detrimental effect of the DS on these parameters was greater in the own-root line than in the corresponding grafted-root line. These results suggest that the *Artemisia annua* rootstock could

https://doi.org/10.17221/177/2022-HORTSCI

improve the drought resistance of chrysanthemum and reduce the dry mass loss under DS. Brassinolide has a positive effect on mitigating the DS by regulating the RWC, $P_N$, Chl fluorescence parameters, Rubisco activity, $H_2O_2$ and AsA-GSH cycle in the own-root and grafted-root chrysanthemums. Brassinolide improved the RWC, $P_N$, $F_v/F_m$, $\Phi_{PSII}$, activity of Rubisco, APX, GR, MDHAR and DHAR, AsA/DHA and GSH/GSSG, and reduced the $H_2O_2$ content in the own-root and grafted-root chrysanthemums under DS. These advantages could alleviate the detrimental effect of the DS on the chrysanthemum growth. As a result, the dry mass of the BL line was significantly higher than that of the corresponding non-BL line under DS, although BL ameliorated the parameters in all the chrysanthemum lines under the DS and WW conditions, and the change in magnitude of the parameters was greater in the own-root line than in the corresponding grafted-root line. The *Artemisia annua* rootstock or BL significantly alleviated the detrimental effects of the DS on the photosynthetic capacity and AsA-GSH cycle in the chrysanthemum, however, those positive effects were more significant when the *Artemisia annua* rootstock and BL were applied together.

## REFERENCES


Ahsan M.U., Hayward A., Alam M., Bandaralage J.H., Topp B., Beveridge C.A., Mitter N. (2019): Scion control of miRNA abundance and tree maturity in grafted avocado. BMC Plant Biology, 19: 382.

Baker N.R. (2008): Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. Annual Review of Plant Biology, 59: 89–113.

Chen L.S., Qi Y.P., Smith B.R., Liu X.H. (2005): Aluminum-induced decrease in $CO_2$ assimilation in citrus seedlings is unaccompanied by decreased activities of key enzymes involved in $CO_2$ assimilation. Tree Physiology, 25: 317–324.

Chen Y., Sun X., Zheng C., Zhang S., Yang J. (2018): Grafting onto *Artemisia annua* improves drought tolerance in chrysanthemum by enhancing photosynthetic capacity. Horticultural Plant Journal, 4: 117–125.

Cheng L., Fuchigami L.H. (2000): Rubisco activation state decreases with increasing nitrogen content in apple leaves. Journal of Experimental Botany, 51: 1687–1694.

Deng Z., Zhang X., Tang W., Oses-Prieto J.A., Suzuki N., Gendron J.M., Chen H., Guan S., Chalkley R.J., Peterman T.K. (2007): A proteomic study of brassinosteroid response in *Arabidopsis*. Molecular & Cellular Proteomics, 6: 2058–2071.

Dias M.C., Brüggemann W. (2007): Differential inhibition of photosynthesis under drought stress in *Flaveria* species with different degrees of development of the $C_4$ syndrome. Photosynthetica, 45: 75–84.

Erb T.J., Zarzycki J. (2018): A short history of Rubisco: the rise and fall (?) of nature's predominant $CO_2$ fxing enzyme. Current Opinion in Biotechnology, 49: 100–107.

Guan Z., Wu D., Song A., Chen F., Chen S., Fang W. (2017): A highly sensitive method for the detection of Chrysanthemum virus B. Electronic Journal of Biotechnology, 26: 64–68.

Hayat S., Ali B., Hasan S.A., Ahmad A. (2007): Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. Environmental and Experimental Botany, 60: 33–41.

Hu H., Shan C. (2018): Effect of cerium (Ce) on the redox states of ascorbate and glutathione through ascorbate-glutathione cycle in the roots of maize seedlings under salt stress. Cereal Research Communications, 46: 31–40.

Hu Y.J., Shi L.X., Sun W., Guo J.X. (2013): Effects of abscisic acid and brassinolide on photosynthetic characteristics of *Leymus chinensis* from Songnen Plain grassland in Northeast China. Botanical Studies, 54: 42.

Huang Y., Jiao Y.Y., Nawaz M.A., Chen C., Liu L., Lu Z., Kong Q.S., Cheng Fei., Bie Z.L. (2016): Improving magnesium uptake, photosynthesis and antioxidant enzyme activities of watermelon by grafting onto pumpkin rootstock under low magnesium. Plant Soil, 409: 229–246.

Janeczko A., Gruszka D., Pociecha E., Dziurka M., Filek M., Jurczyk B., Kalaji H.M., Kocurek M., Waligórski P. (2016): Physiological and biochemical characterisation of watered and drought-stressed barley mutants in the HvDWARF gene encoding C6-oxidase involved in brassinosteroid biosynthesis. Plant Physiology and Biochemistry, 99: 126–141.

Khamsuk O., Sonjaroon W., Suwanwong S., Jutamanee K., Suksamrarn A. (2018): Effects of 24-epibrassinolide and the synthetic brassinosteroid mimic on chili pepper under drought. Acta Physiologiae Plantarum, 40: 106.

Lee J.M. (1994): Cultivation of grafted vegetables I. Current status, grafting methods, and benefits. Hortscience, 29: 235–239.

Li Y., Wu Y., Liao W., Hu L., Dawuda M.M., Jin X., Tang Z., Yang J., Yu J. (2020): Nitric oxide is involved in the brassinolide-induced adventitious root development in cucumber. BMC Plant Biology, 20: 102.

Liu J.P., Sun X.J., Xu F.Y., Zhang Y.J., Zhang Q., Miao R., Zhang J.H., Liang J.S., Xu W.F. (2018): Suppression of *OsMDHAR4* enhances heat tolerance by mediating $H_2O_2$-induced stomatal closure in rice plants. Rice, 11: 38.

Noctor G., Mhamdi A., Foyer C.H. (2016): Oxidative stress and antioxidative systems: recipes for successful data col-

https://doi.org/10.17221/177/2022-HORTSCI

lection and interpretation. Plant Cell and Environment, 39: 1140–1160.

Rao M.V., Ormrod D.P. (1995): Ozone exposure decreases UVB sensitivity in a UVB-sensitive flavonoid mutant of Arabidopsis. Photochemistry and Photobiology, 61: 71–78.

Sadura I. and Janeczko A. (2018): Physiological and molecular mechanisms of brassinosteroid-induced tolerance to high and low temperature in plants. Biologia Plantarum, 62: 601–616.

Sang Q.Q., Shu S., Shan X., Guo S.R., Sun J. (2016): Effects of exogenous spermidine on antioxidant system of tomato seedlings exposed to high temperature stress. Russian Journal of Plant Physiology, 63: 645–655.

Sun J., Gu J., Zeng J., Han S., Song A.P., Chen F.D., Fang W.M., Jiang J.F., Chen S.M. (2013): Changes in leaf morphology, antioxidant activity and photosynthesis capacity in two different drought-tolerant cultivars of chrysanthemum during and after water stress. Scientia Horticulturae, 161: 249–258.

Sun J.S., Hu R.Y., Lv F.L. Yang Y.F., Tang Z.M., Zheng G.S., Li J.B., Tian H., Xu Y., Li S.F. (2020): Comparative transcriptome analysis reveals stem secondary growth of grafted *Rosa rugosa* 'Rosea' scion and *R. multiflora* 'Innermis' rootstock. Genes, 11: 228.

Tanveer M., Shahzad B., Sharma A., Biju S., Bhardwaj R. (2018): 24-Epibrassinolide; an active brassinolide and its role in salt stress tolerance in plants: a review. Plant Physiology and Biochemistry, 130: 69–79.

Teuling A.J. (2018): A hot future for European droughts. Nature Climate Change, 8: 364–365.

Ueda Y., Wu L., Frei M. (2013): A critical comparison of two high-throughput ascorbate analyses methods for plant samples. Plant Physiology and Biochemistry, 70: 418–423.

Xia X.J., Huang L.F., Zhou Y.H., Mao W.H., Shi K., Wu J.X., Asami T., Chen Z., Yu J.Q. (2009): Brassinosteroids promote photosynthesis and growth by enhancing activation of Rubisco and expression of photosynthetic genes in *Cucumis sativus*. Planta, 230: 1185–1196.

Yousuf P.Y., Ahmad A., Ganie A.H., Sareer O., Krishnapriya V., Aref I.M., Iqbal M. (2017): Antioxidant response and proteomic modulations in Indian mustard grown under salt stress. Plant Growth Regulation, 81: 31–50.

Zhang A., Liu M., Gu W. Chen Z., Gu Y., Pei L. & Tian R. (2021): Effect of drought on photosynthesis, total antioxidant capacity, bioactive component accumulation, and the transcriptome of *Atractylodes lancea*. BMC Plant Biology, 21: 293.

Zhang X.Y., Sun X.Z., Zhang S., Yang J.H., Liu F.F., Fan J. (2019): Comprehensive transcriptome analysis of grafting onto *Artemisia scoparia* W. to affect the aphid resistance of chrysanthemum (*Chrysanthemum morifolium* T.). BMC Genomics, 20: 776.

Received: December 26, 2022
Accepted: July 29, 2023
Published online: January 16, 2024